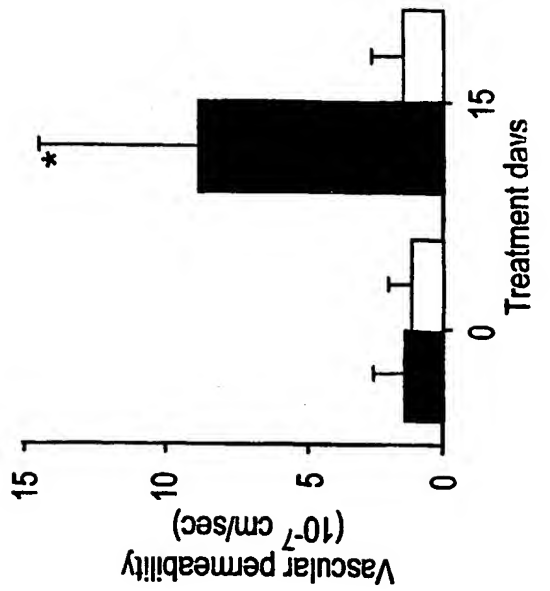
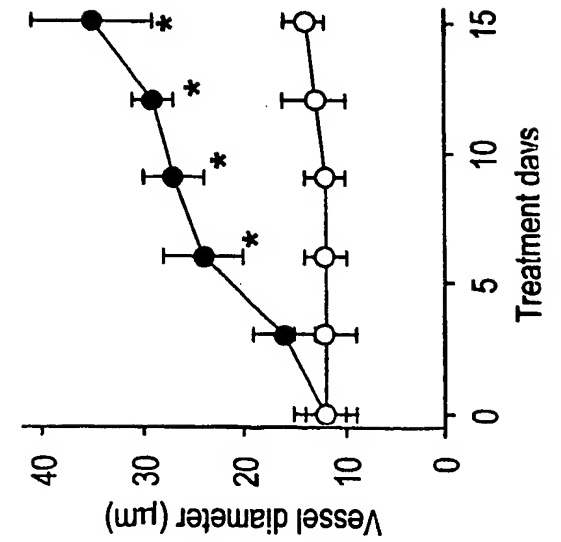


FIG. 1B



2/19

FIG. 1E

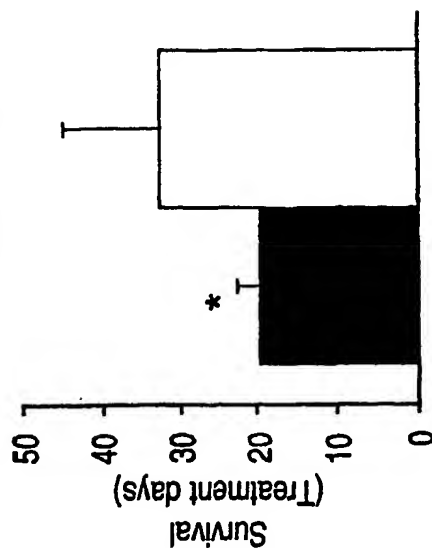


FIG. 1D

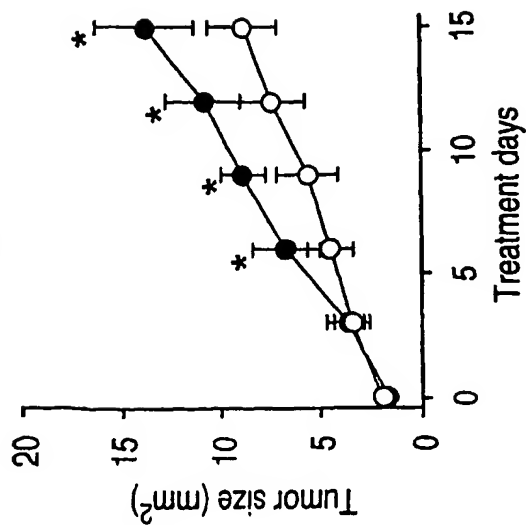


FIG. 1F

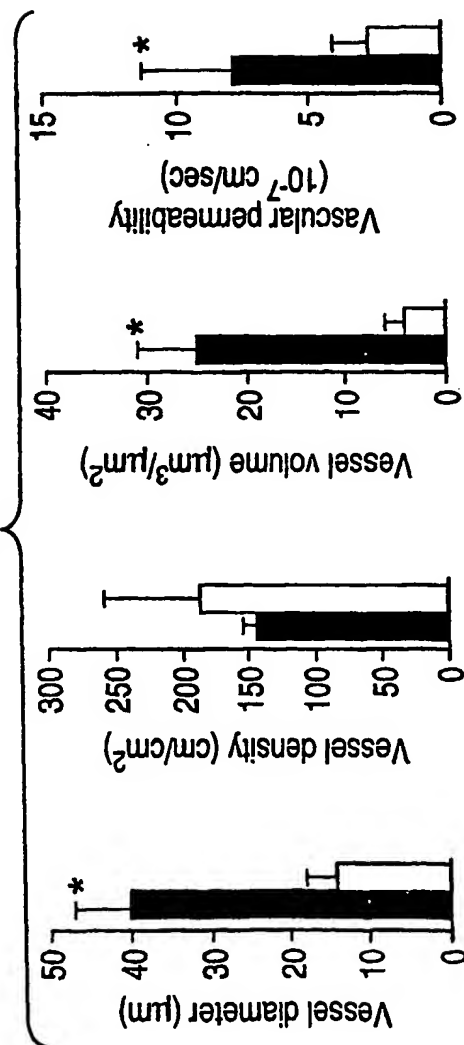


FIG. 1G

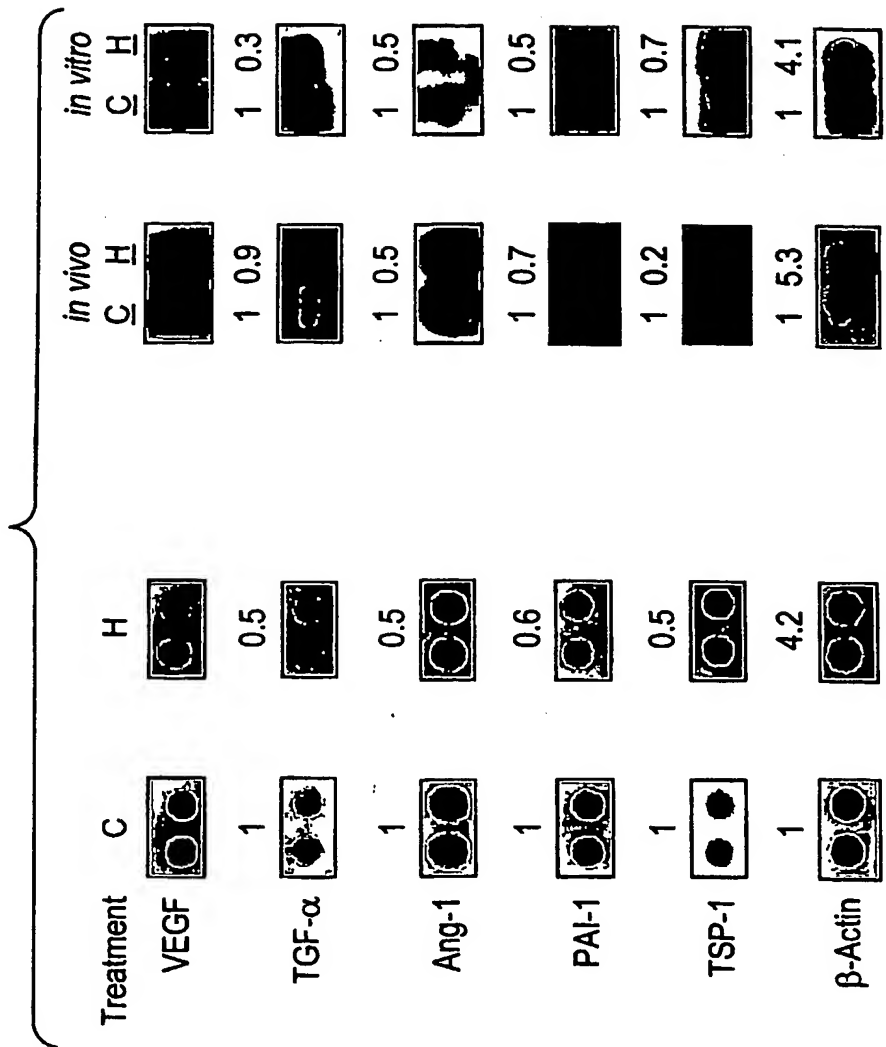
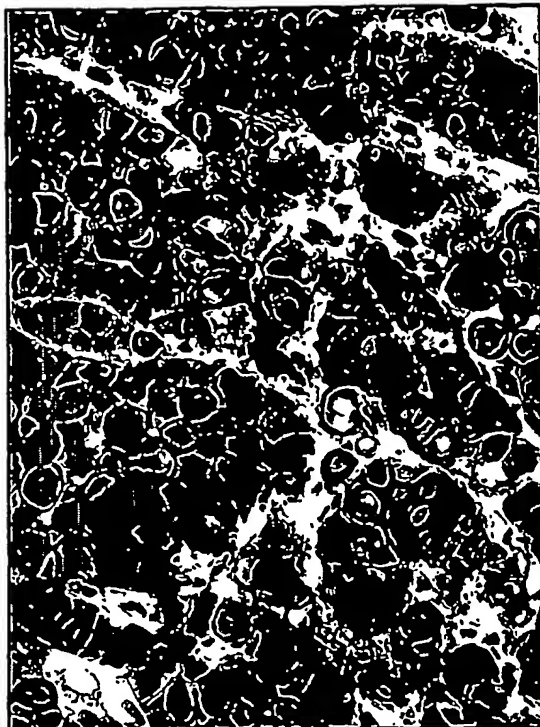


FIG. 1I



VEGF

FIG. 1H



HER2

10/507352

5/19

FIG. 1J

| | Vessel diameter (μm) | Vessel density (cm/cm^2) | Vessel volume ($\mu\text{m}^3/\mu\text{m}^2$) | Permeability (10^{-7} cm/s) | Survival (days) |
|---------------------------|--------------------------------------|---|--|--|--------------------|
| Control | 40.4 ± 6.7 | 144 ± 11 | 25.4 ± 6.3 | 8.0 ± 3.4 | 20 ± 3 |
| Herceptin | $14.2 \pm 4.1^*$ | 181 ± 70 | $3.8 \pm 2.3^*$ | $2.7 \pm 1.4^*$ | $33 \pm 12^*$ |
| Gene expression | VEGF | TGF α | Ang-1 | PAI-1 | TSP-1 |
| Gene array <i>in vivo</i> | 0.5 | 0.5 | 0.6 | 0.5 | 4.2 |
| Northern <i>in vivo</i> | 0.9 | 0.5 | 0.7 | 0.2 | 5.3 |
| Northern <i>in vitro</i> | 0.3 | 0.5 | 0.5 | 0.7 | 4.1 |

10/507352

6/19

FIG. 2A

Angiogenesis inhibitors in clinical trials for cancer

| Drug | Mechanism |
|----------------------------|--|
| Phase I | |
| EMD121974 | Small molecule integrin antagonist |
| Combretastatin A-4 prodrug | Apoptosis in proliferating endothelium |
| PTK787/ZK2284 | Blocks VEGF-receptor signaling |
| Endostatin | Induces endothelial cell apoptosis <i>in vivo</i> |
| BMS-275291 | Synthetic MMP inhibitor |
| SU6668 | Blocks VEGF-, FGF-, and PDGF- receptor signaling |
| Phase II | |
| CAI | Inhibitor of calcium influx |
| Squalamine | Inhibits Na ⁺ /H ⁺ exchanger |
| COL-3 | Synthetic MMP inhibitor; tetracycline derivative |
| CGS-27023A | Synthetic MMP inhibitor |
| TNP-470 | Fumagilin analogue; inhibits endothelial proliferation |
| Vitaxin | Antibody to integrin on endothelial surface |
| IL-12 | Induces interferon- γ and IP-10 |
| Anti-VEGF Ab | Monoclonal antibody to VEGF |
| Phase III | |
| SU5416 | Blocks VEGF receptor signaling |
| Thalidomide | Unknown |
| Marimastat | Synthetic MMP inhibitor |
| AG3340 | Synthetic MMP inhibitor |
| Neovastat | Natural MMP inhibitor |
| Interferon- α | Inhibition of bFGF and VEGF production |
| IM862 | Unknown mechanism |

From NCI Database www.cancertrials.nci.nih.gov (updated 12 April 2000)

10/507352

7/19

FIG. 2B

| Farnesyltransferase Inhibitors in Clinical Development | | |
|---|----------------------------|-------------------------|
| Farnesyltransferase Inhibitor | Route of Administration | Phase of Development |
| R115777 | Oral | Phase III |
| BMS-214662 | Oral or IV | Phase I |
| SCH6636 | Oral | Phase II |
| L-778,123 | IV | Phase I |

10/507352

8/19

FIG. 2C

| Completed and Active Clinical Studies With INGN 201 | | | |
|--|-------------------------------------|--|--|
| Phase | Disease | Route of Administration Of Gene Therapy | Study Status |
| I | Head and neck | IT | Completed (no MTD) ²⁰ |
| | Non-small cell lung | IT | Completed (no MTD) ^{23,24} |
| | Prostate | I Pros | Completed (no MTD) ²⁷ |
| | Solid tumors | IV | Ongoing |
| | Ovarian | IP | Ongoing (2 studies) ²⁸ |
| | Breast | IT | Ongoing |
| | Bladder | I Vesc | Ongoing |
| | Brain | IT | Ongoing ²⁹ |
| | Lung | BAL | Ongoing |
| | Head and neck | IT | Completed (2 studies) ²¹ |
| II | Non-small cell lung | IT | Ongoing ²⁵ |
| III | Head and neck (single agent) | IT | Ongoing |
| | Head and neck (+ cisplatin/5-FU) | IT | Ongoing |
| Abbreviations: IT, intratumoral; I Pros, Intraprostatic; IV, Intravenous; IP, Intraperitoneal; I Vesc, intravesical Instillation; BAL, bronchopulmonary lavage; MTD, maximum tolerated dose; 5-FU, 5-fluorouracil. | | | |

9/19

10/507352

FIG. 2D

| Potential Indications, Adverse Events, and Trial Status of Drugs Targeting the ErbB Receptor | | |
|--|---|---|
| Drug | Potential Indications | Major Adverse Events |
| Monoclonal Antibodies | | |
| Trastuzumab | ErbB2-overexpressing metastatic breast cancer (FDA approved); Other erbB2-driven tumors | Fever, chills, pain, dyspnea; cardiotoxicity, especially when combined with cytotoxic drugs |
| C225 | EGFR-driven tumors, especially head and neck | Fever, chills, asthenia, nausea, acneiform, rash |
| MDX-H210 | erbB2-driven tumors | Acute reactions to IV infusion |
| MDX-447 | EGFR-driven tumors | Hypotension |
| Tyrosine Kinase Inhibitors | | |
| ZD1839 | EGFR-driven tumors, especially NSCLC | Rash, diarrhea, nausea, vomiting |
| OSI-774 | EGFR-driven tumors, especially NSCLC | Fatigue, headache, nausea, diarrhea, rash |
| CI-1033 | Tumors driven by any one or multiple erbB receptors | NA |
| PK1-166 | EGFR-driven tumors | NA |
| Abbreviations: NSCLC, non-small cell lung cancer; IV, intravenous; FDA, Food and Drug Administration | | |

10/19

FIG. 2E

| Small-Molecule Inhibitors of the EGFR Kinase Currently in Preclinical and Clinical Development | | | |
|--|--|---|--------------|
| Small Molecule | EGFR IC ₅₀ (μ mol/L)* | HER2 IC ₅₀ (μ mol/L) | Reference(s) |
| AG-1478 | <0.003 | 1.4+ | 5.25 |
| AG-1517¶ | 0.0009 | Not reported | 25 |
| PD153035¶ | 0.029 \pm 0.005 | 2.3+ | 28 |
| ZD1839 | 0.033 | >3.7++ | 29 |
| OSI-774 | 0.02 | Not reported | 30 |
| PD168393# | 0.0007 \pm 0.00009 | 5.7 \pm 0.8§ | 23 |
| PD158780 | 0.000008 | 0.05 | 24 |
| <p>*Most values reflect the IC₅₀ using purified EGFR in vitro as a substrate +Personal communication, Laura Shawver, Sugen, Inc (South San Francisco, CA), 1998. ++Effect purified HER2 kinase in vitro §Effect on heregulin-mediated phosphorylation (of HER2). Effect on heregulin-stimulated phosphorylation (of HER2) in SKBR-3 and MDA-453 cells. ¶These two quinazolines have the same structure (refs 4 and 28) #Only reported irreversible inhibitor.</p> | | | |

10/507352

11/19

FIG. 2F-1

| Drugs that block matrix breakdown: | | | | |
|--|---|---|--|--|
| Drug | Sponsor | Trial | Mechanism | For More Info: |
| Marimastat | British Biotech | Phase III small cell lung cancers | Synthetic inhibitor of matrix metalloproteinases (MMPs) | 800-4-CANCER Online Information |
| COL-3 | Collagenex; Newtown, PA | Phase I/II brain, Kaposi's Sarcoma | Synthetic MMP inhibitor Tetracycline® derivative | 800-4-CANCER Online Information |
| Neovastat | Aeterna; Québec | Phase II Multiple Myeloma, Phase III renal cell (kidney) cancer, Phase III non-small cell lung cancer | Naturally occurring MMP inhibitor | 888-349-3232 Online Information |
| BMS-275291 | Bristol-Myers Squibb; Wallingford, CT | Phase I/II Kaposi's sarcoma, Phase II/III Advanced or Metastatic Non-Small Cell Lung | Synthetic MMP inhibitor | 203-677-6779 Online Information |
| Drugs that inhibit endothelial cells directly: | | | | |
| Drug | Sponsor | Trial | Mechanism | For More Info: |
| Thalidomide | Commercially available, approved for leprosy, Celgene | Phase I Malignant Glioma, Phase I/II for advanced Melanoma, Phase II ovarian, metastatic prostate, Phase II with chemotherapy against solid tumors; adjuvant study in recurrent or metastatic colorectal cancer. Myelofibrosis with myeloid metaplasia, follicular lymphoma, myelodysplastic syndrome, refractory ovarian, Phase II gynecologic sarcomas, liver cancer, metastatic melanoma, CLL, Multiple Myeloma, Phase III non-small cell lung, nonmetastatic prostate, refractory multiple myeloma, renal cancer. | Unknown | 732-805-3905 or 800-890-4619 ext. 3905 or 800-4-CANCER or 1-888-NCI-1937 Online Information |
| Squalamine | Genera Pharmaceuticals; Plymouth Meeting, PA | Phase II non small cell lung cancer; Phase II Ovarian; Brain; Phase I Advanced Cancers | Extract from dogfish shark liver; inhibits sodium-hydrogen exchanger, NHE3 | 610-841-4020 or 800-4-CANCER Online Information |
| 2-ME | EntireMed, Rockville, MD | Phase I solid tumor studies | Inhibition of endothelial cells | 800-4-CANCER Online Information |

10/507352

12/19

FIG. 2F-2

| Drugs that block activators of angiogenesis: | | | | |
|--|---|--|--|--|
| Drug | Sponsor | Trial | Mechanism | For More Info: |
| SU6668 | Sugen, South San Francisco, CA | Phase I against advanced tumors | Blocks VEGF, FGF, and PDGF receptor signaling | 800-SUGEN-06 or 650-553-8878 Online Information (Link to Sugen Web site) |
| Interferon-alpha | Commercially available | Phase II/III (search* NCI trials database for listings) | Inhibition of bFGF and VEGF production | 800-4-CANCER or 888-NCI-1937 |
| Anti-VEGF Antibody | National Cancer Institute, Bethesda, MD; Genentech, San Francisco, CA | Advanced head and neck. Phase II metastatic renal cell cancer, Phase II with chemotherapy in untreated advanced colorectal, metastatic breast; Phase II non-hodgkin's lymphoma, hematologic malignancies, metastatic prostate, previously untreated advanced colorectal, inflammatory breast cancer, Advanced or recurrent cervical, non-small cell lung; Phase II/III Advanced non-small cell lung; Phase III with chemotherapy in untreated metastatic colorectal, Phase III metastatic breast | Monoclonal antibody to vascular endothelial growth factor (VEGF) | 888-624-1937 or 800-4-CANCER Online Information |

* When searching for interferon-alpha trials in the NCI database, select "cytokine therapy" rather than "antiangiogenesis therapy" in the modality field.

| Drugs that inhibit endothelial-specific integrin/survival signaling: | | | | |
|--|---|--|--|---|
| Drug | Sponsor | Trial | Mechanism | For More Info: |
| Medi-522 (Vitaxin II) | MedImmune, Inc., Gaithersburg, Maryland | Phase I/II trial in CPT-11 (irinotecan) refractory advanced colorectal cancer | Antibody that blocks the integrin present on endothelial cell surface | 800-4-CANCER Online Information |
| EMD121974 | Merck KGaA; Darmstadt, Germany | Phase I in patients with HIV related Kaposi's Sarcoma, Phase I/II progressive or recurrent Anaplastic Glioma | Small molecule blocker of integrin present on endothelial cell surface | 800-4-CANCER Online Information |

13/19

10/507352

FIG. 2F-3

Drugs with non-specific mechanism of action:

| Drug | Sponsor | Trial | Mechanism | For More Info: |
|-----------------------------|---|--|---|--|
| irinotecan | National Cancer Institute, Bethesda, MD | Phase I studies in combination against solid tumors, Phase II ovarian cancer, metastatic renal cell cancer | Inhibitor of calcium influx | 800-4-CANCER or 888-624-1937 <u>Online information</u> |
| cyclooxygenase 2 inhibitors | Pharmacia | Phase I Prostate; Phase I/II Cervical; Phase II Basal Cell, Metastatic Breast | Enzyme cyclo-oxygenase 2 (COX-2) | 800-4-CANCER <u>Online information</u> |
| interleukin-12 | Genetics Institute; Cambridge, MA | Phase I/II Kaposi's sarcoma | Up-regulation of interferon gamma and IP-10 | 800-4-CANCER <u>Online information</u> |
| irinotecan | Cytran; Kirkland, WA | Phase II for untreated metastatic cancers of the colon and rectum; Ovarian | Unknown mechanism | 425-889-5808 or 800-4-CANCER <u>Online information</u> |

10/507352

14/19

FIG. 2G-1

Tyrosine kinase inhibitor

1. ZD1839
2. Cetuximab
3. STI571
4. SU5416
5. SU6668
6. Phenoxodiol
7. Imatinib Mesylate
8. Erlotinib
9. OSI-774
10. USN-01

Enzyme inhibitor

1. PS-341
2. ISIS3521
3. AG2037
4. Imatinib Mesylate
5. STI571
6. ZD1839
7. R115777
8. SCH66336
9. Phenoxodil
10. SU5416
11. Celecoxib
12. Erlotinib
13. Trastuzumab
14. OSI-774
15. Paclitaxel, Lometrexol

Other signaling inhibitors

1. Perifosine: alkylphospholipid modulator of signal transduction
2. Flavopiridole: cyclin-dependent kinase inhibitor
3. Genasense (G3139): Bcl-2 antisense
4. Ras peptide vaccine
5. P53 peptide vaccine
6. VHL peptide

10/507352

15/19

FIG. 2G-2

Antibody therapy

1. BEC2
2. CD20 (Rituximab)
3. ch14.18
4. CD52 (Campath-1H)
5. ABX-CBL
6. Edrecolomab
7. Trastuzumab
8. m170
9. Lym-1
10. BrE-3
11. M195
12. Bevacizumab: rhuMAb VEGF
13. Tositumomab
14. 3F8
15. HMFG1
16. CC49-deltaCH2
17. IDEC-Y2B8 (Ibritumomab tiuxetan)
18. IDEC-In2B8
19. Hu3S193
20. HeFi-1
21. 81C6
22. Hu1D10 (Apolizumab)
23. ABX-EGF
24. HuM291
25. 4G7xH22
26. MN-14
27. huJ591
28. 105AD7
29. SGN-15 (cBR96-doxorubicin
immunoconjugate)
30. Gemtuzumab ozogamicin
31. MDX-CTLA4
32. Zenapax (daclizumab, anti-Tac):
anti-IL2 receptor alpha
33. Cetuximab
34. OKT3
35. Epratuzumab
36. TNT-1/B
37. MDX447
38. IL-13 PE38QQR immunotoxin
39. LMB-9 immunotoxin
40. MIK-Beta-1
41. I131 anti-B1 antibody
42. Cereport: anti-brain capillary
endothelial cell B2 receptor

10/507352

16/19

FIG. 2G-3**Hormonal**

1. Letrozole: antiestrogen
2. Anastrozole: endocrine therapy
3. Raloxifene: anti-estrogen
4. Medroxyprogesterone: progesterone therapy
5. Bicalutamide: anti-androgen

Cytokine related

1. Anti-thymocyte globulin and TNF receptor IgG chimera: anti-cytokine, biological response modifier
2. Filgrastim (G-CSF): cytokine therapy
3. Enbrel (Tumor necrosis factor fusion protein): anti-cytokine
4. IL13-PE38QQR: IL13 + PE38QQR (bacteria toxin), cell kill against IL13 positive tumor cells
5. Etanercept: soluble TNF alpha receptor, anti-inflammatory
6. Interferon alpha: inhibit renal cell growth, immunotherapy
7. F1t3L: cytokine therapy
8. Sargramostim: cytokine
9. Infliximab: anti-TNF alpha
10. Azacitidine: cytokine
11. Amifostine: cytokine

Growth factor antagonist

1. Temozolomide
2. Deltaparin
3. EMD121974
4. CC-5013: Thalidomide analogue
5. RPI4610
6. Shark cartilage extract

Immune modifier

1. APC8015
2. BMS-275291

10/507352

17/19

FIG. 2G-4

Chemosensitizer

1. PSC 833: drug resistance inhibition
2. Bryostat 1: chemosensitizer
3. UCN-01: decrease tumor threshold for apoptosis

Chemoprevention

1. Eflornithine: chemoprevention
2. Sulindac: chemoprevention
3. LY353381

Others

1. Goserelin: releasing factor agonist
2. Exemestane: aromatase inhibition
3. Tretinoin: normalize renal cancer cells

10/507352

18/19

FIG. 3

Angiogenesis activators and inhibitors

| Activators | Function | Inhibitors | Function |
|---|---|--|--|
| VEGF family Members (+) (#) | Stimulate angio / vasculogenesis, permeability, leukocyte adhesion | VEGFR-1; soluble VEGFR-1 soluble neuropilin-1 (NRP-1) | Sink for VEGF, VEGF-B, PlGF |
| VEGFR (#), NRP-1, NRP-2 | Integrate angiogenic and survival signals | Ang2(#)(*) | Antagonist of Ang1 |
| EG-VEGF | Stimulate growth of endothelial cells derived from endocrine glands | TSP-1,2 | Inhibit endothelial migration, growth, adhesion & survival |
| Ang1 and Tie2 (+)(#) | Stabilize vessels | Angiostatin and related plasminogen kringle | Inhibit endothelial migration and survival |
| PDGF-BB and Receptors | Recruit smooth muscle cells | Endostatin (Collagen XVIII fragment) | Inhibit endothelial survival and migration |
| TGF- β 1 (*), endoglin, TGF- β receptors | Stimulate extracellular matrix production | Tumstatin | Inhibit endothelial protein synthesis |
| FGF, HGF, MCP-1 | Stimulate angio/arteriogenesis | Vasostatin; calreticulin | Inhibit endothelial growth |
| Integrins α v β 3(*), α v β 5, α β 1 | Receptors for matrix macromolecules and proteinases | Platelet factor-4 | Inhibit binding of bFGF and VEGF |
| VE-cadherin; PECAM (CD31) | Endothelial junctional molecules | Tissue-inhibitors of MMP (TIMPs); MMP-inhibitors; PEX | Suppress pathologic angiogenesis |
| Ephrins (#) | Regulate arterial / venous specification | Meth-1, Meth-2 | Inhibitors containing MMP-, TSP-, and disintegrin-domains |
| Plasminogen Activators, MMPs | Remodel matrix, release growth factor | IFN- α , - β , - γ , IP-10, IL-4, IL-12, IL-18 | Inhibit endothelial migration; downregulate bFGF |
| PAI-1 | Stabilize nascent vessels | Prothrombin kringle-2; anti-thrombin III fragment | Suppress endothelial growth |
| NOS; COX-2 | Stimulate angiogenesis and vasodilation | 16 kD-prolactin | Inhibit bFGF/VEGF |
| AC133 | Regulate angioblast differentiation | VEGI | Modulate cell growth |
| Chemokines (*) | Pleiotropic role in angiogenesis | Fragment of SPARC | Inhibit endothelial binding and activity of VEGF |
| Id1/Id3 | Inhibit differentiation | Osteopontin fragment | Interfere with integrin signaling |
| | | Maspin | Protease inhibitor |
| | | Canstatin, Proliferin-related protein, Restin | Mechanisms unknown |

For complete function and references, see supplementary information (<http://steele.mgh.harvard.edu>);
 (*): opposite effect in some contexts; (+): also present in or affecting non-endothelial cells.

FIG. 4

| Angiogenesis in neoplasms and other diseases | | | |
|--|---|--|---|
| Organ | Processes characterized by abnormal angiogenesis or vascular malfunction* | Organ | Processes characterized by abnormal angiogenesis or vascular malfunction* |
| Cardiovascular | †Atherosclerosis, haemangioma, haemangioendothelioma, §Vascular malformations | Bone, joints | †Rheumatoid arthritis, synovitis, bone and cartilage destruction osteomyelitis, pannus growth, osteophyte formation, cancer ‡Aseptic necrosis, impaired healing of fractures |
| Brain, nerves, eye | †Warts, pyogenic granulomas, hair growth, Kaposi's sarcoma, scar keloids, allergic oedema, neoplasms §Psoriasis (skin vessels enlarge and become tortuous) ‡Decubitus or stasis ulcers, gastrointestinal ulcers | Liver, kidney, lung, ear and other epithelia | †Inflammatory and infectious processes (hepatitis, pneumonia glomerulonephritis), asthma, nasal polyps, transplantation, liver regeneration, cancer §Pulmonary hypertension, diabetes ‡Pulmonary and systemic hypertension (vascular pruning) |
| Endocrine organs | †Dysfunctional uterine bleeding (contraception), follicular cysts, ovarian hyperstimulation, endometriosis, neoplasms §Pre-eclampsia ‡Placental insufficiency | Brain, nerves, eye | †Retinopathy of prematurity, diabetic retinopathy, choroidal and other intraocular disorders, leukomalacia, cancer ‡Stroke vascular dementia, Alzheimer's disease, CADASIL |
| Endocrine organs | Respiratory distress, ascites, peritoneal sclerosis (dialysis patients), adhesion formation (abdominal surgery), metastatic spreading | Endocrine organs | †Thyroiditis, thyroid enlargement, pancreas transplantation ‡Thyroid pseudocyst |
| Lymph vessels | †Work overload ‡Ischaemic heart and limb disease | Lymph vessels | †Tumor metastasis, lymphoproliferative disorders ‡Lymphoedema |
| Haematopoiesis | †Obesity | Haematopoiesis | †AIDS (Kaposi), haematologic malignancies |

† of selected examples

||| increased vascularization; § abnormal remodeling; || increased vascularization and/or permeability; see text for abbreviations